0273-2289/92/32(1/3)—0023$02.60

Actinomycetes in Submerged Culture

ALLAN WHITAKER

Division of Biosciences, Hatfield Polytechnic, College Lane, Hatfield, AL10 9AB UK

Received October 14, 1991; Accepted October 25, 1991

ABSTRACT

The range of morphological forms of actinomycetes in shaken flask culture and fermenters is reviewed. Some of the factors that influence pellet formation and its prevention are discussed. The relationship between morphology and production of antibiotics and other metabolites is also examined.

Index Entries: Actinomycetes; *Streptomyces;* pellets; mycelial aggregates; disperse mycelium; fragmented mycelium; pellet initiation; inoculum; growth conditions; pellet prevention; antibiotics.

INTRODUCTION

The information available about the morphology of actinomycetes grown in submerged culture is restricted even though this group of microorganisms is used to produce many of the antibiotics in current use. Nevertheless, a number of aspects of growth forms have been investigated. They may depend on the actinomycete species, the individual strain, the size of the spore inoculum, the growth medium, or the physical environment within the culture vessel. Growth forms may vary from compact pellets of mycelium of 5 mm diameter to suspensions of fragmented hyphae that have a bacillary form. In contrast, there are many reports on fungal morphology (*1,2*).

*Author to whom all correspondence and reprint requests should be addressed.

GROWTH FORMS

When the gross morphology of actinomycetes is described in literature, it is often very vague and open to different interpretations. Descriptions are very rarely supported by photographs or diagrams to illustrate what is actually present. Recently, a semiautomated image analyzer has been developed for morphological measurements of mycelial *Streptomyces* strains that may overcome some of the problems (*3*).

One of the earliest descriptions of morphology was made by Erikson and Porteous, who described the anaerobic actinomycete *Actinomyces israelii* as having compact "bread crumb," "cauliflower," or "puff ball" colonies that settled out in static culture (*4*). Waksman reported small, bead-like colonies, granules, or flakes of actinomycetes grown in submerged culture (*5,6*). Tresner et al. examined 145 *Streptomyces* species (sp) and found a continuum of morphological types that ranged from mycelial pellets several mm in diameter to fragmented forms (*7*). Williams et al. reported loose, open colonies and free, hyphal colonies with dense central regions, radiating hyphae, and hyphal ropes (*8*). Lawton et al. examined 49 *Streptomyces* strains and eight other actinomycetes using a range of spore inocula (*9*). They found that each strain would produce one or more morphological forms that included compact pellets, spiky or fluffy pellets, oblong pellets, flakes, hydrophobic rafts, aggregates, dispersed mycelium, or fragmented mycelium and gave photographs of the different growth forms. Good descriptions have also been given by Blumauerova et al., Al-Jawadi and Calam, and Cizmek and Drazic (*10–12*).

Much of the published information about growth forms is summarized in Table 1 for shaken flask cultures that normally only form pellets (A); only form mycelial aggregates or fragmented mycelium (B); or form pellets and a range of mycelial forms (C). Growth forms in a range of fermenters are given in Table 2. Most of the data has been obtained with one inoculum level except for the work of Lawton et al., Hobbs et al., and Vecht-Lifshitz et al. (*9,13,14*).

Initial Stages of Pellet Formation

Two mechanisms of pellet formation can be recognized. In an early study of *S. griseus,* branched hyphae from germinating spores entangled to form a loose weft (*38,48*). Further branching then took place, and the central cores of the pellets became denser. Active hyphal growth was confined to the surface of older pellets. With aging, the central core may lyse, with breakup starting after 3–4 d. Kolstad and Bradley observed *S. venezuelae* spores germinating after 2 h incubation and mycelia clustering together after 10 h (*73*). Similar development processes have been reported with *S. aureofaciens, S. tendae,* and *Micromonospora vulgaris* (*7,14,74*).

Table 1A
Growth Forms in Shake Flasks of Organisms Forming Pellets Only

Strain	Morphological form	Reference
Actinomadura madurae	Compact spheres	9
Actinomyces israelii	Compact "bread crumbs, cauliflowers, or puff balls"	4
Streptomyces alboduncus	Compact spheres	9
S. arenae	Compact spheres	9
S. armentosus	Spiky, fluffy pellets	9
S. calvus	Compact spheres	9
S. cinnamensis	Spiky, fluffy pellets	9
S. ederensis	Spike, fluffy pellets	9
S. felleus	Compact and irregular pellets	15
S. fungicidus	Compact pellets	9
S. halstedii	Flakes	9
S. harbonensis	Flakes	9
S. heteromophus	Mixture of compact, spiky, and multi pellets	9
S. lividans	Not described	16
S. melanchromogenes	Not described	8
S. noursei	Not described	8
S. panagensis	Compact spheres	9
S. phaeochromogenes	Flakes	9
S. scabies	Not described	17
S. tendae	Spherical pellets	14
S. violaceoruber	Not described	18
Streptomyces J-13-3	Dense centers and open margins	19
Thermomonospora fusca	Fluffy pellets	20

An alternative mechanism for *S. viridochromogenes* and a *Streptosporangium* sp. involves the initial clumping or coagulation of spores that then germinate to produce hyphae, which gradually become entangled and develop into pellets (*75,76*).

Vecht-Lifshitz et al. think that there may be hydrophobic interactions of cell surfaces that result from the release of bioflocculants, which are biologically regulated in the growth medium in response to oxygen availability (*14*).

Inoculum Size

It is evident that the morphological form of a number of actinomycetes is influenced by the concentration of viable spores in the inoculum. A high concentration tends to produce a disperse form of growth, whereas a low concentration normally results in pellet formation.

Table 1B
Growth Forms in Shake Flasks
of Organisms Forming Only Mycelial Aggregates or Fragmented Mycelium

Strain	Morphological form	Reference
Geodermatophilus sp.	Aggregate of coccoid-budding or motile-budding cells	21
Micromonospora sp.	Hydrophobic rafts	9
Mycobacterium sp.	Hydrophobic rafts	9
M. fortiutum	Cells	22
Nocardia sp.	Mycelium/rods/cocci	23
N. asteroides	Filaments/rods	24
N. erythropolis	Mycelium/fragments	25
N. restricta	Rods/cocci	26
N. rubra	Filaments/bacilli	27
Streptomyces acrimycini	Mycelial aggregates	28
S. cinereoruber	Mycelial aggregates	19
S. clavuligerus	Mycelium	3
S. distallicus	Fragmented mycelium	9
S. echinatus	Mycelium	18
S. espinosus	Mycelium	29
S. fradiae	Fragmented mycelium	9
S. fragmentans	Bacilli	30
S. granaticolar	Mycelium/cocci	31
S. griseofuscus	Mycelial aggregates	32
S. hydroscopicus	Mycelium/fragments	33
S. jamaicensis	Fragmented mycelium	34
S. kasugaensis	Fragmented mycelium	9
S. lactamdurans	Fragmented mycelium	9
S. niveus	Filaments	35
S. rochei	Mycelium	36
S. showdensis	Fragmented mycelium	9
Streptomyces sp.	Mycelium	37

A limited study of *S. aureofaciens* was made by Tresner et al., but they did not estimate initial spore concentrations. When the volume of inoculum was increased from 1 to 15%, there was a higher degree of mycelial fragmentation (*7*).

In a detailed study using a tenfold range of spore dilutions, Lawton et al. found that some actinomycetes always pelleted, others always produced disperse growth forms, and many others formed two growth forms depending on the initial spore concentrations (*9*). *S. vinaceous* and a strain of *S. viridochromogenes* produced compact pellets, spiky/oblong pellets, or disperse mycelium.

Hobbs et al. have investigated the effect of spore inoculum size of *S. coelicolor* A3 on biomass and actinorhodin production (*13*). They

Table 1C
Growth Forms in Shake Flasks
of Organisms Forming Pellets and a Range of Mycelial Forms

Strain	Morphological form	Reference
Streptomyces alboniger	Pellets to aggregates	9
S. antibioticus	Pellets and aggregates	9
S. atrofaciens	Pellets to aggregates	9
S. aureofaciens	Pellets, mycelium, pellets to mycelium and fragmented mycelium	7–9,38–41
S. bambergiensis	Pellets, pellets to pellets, and mycelial aggregates	9,12
S. canadensis	Pellets and disperse mycelium	9
S. cattleya	Pellets to mycelial aggregates	9
S. chartreusis	Pellets to mycelial aggregates	9
S. chrestomyceticus	Pellets and disperse mycelium	9
S. clavuligerus	Pellets and mycelial aggregates	9
S. coelicolor	Pellets and mycelial aggregates	28,42,43
S. erythreus	Pellets	8
	Mycelium	44
S. fimbriatus	Pellets to pellets/mycelial aggregates	9
S. flavogriseus	Pellets to pellets/mycelium	9
S. fradiae	Pellets to mycelial aggregates	9
S. galilaeus	Pellets (reciprocating shaker)	45
	Mycelium (rotary shaker)	45
S. gedaenensis	Pellets to mycelium	9
S. geyseriensis	Pellets to pellets and mycelial aggregates	9
S glomeratus	Pellets and mycelial aggregates	46,47
S. griseus	Pellets, pellets to flocculent growth, pellets to intermingling mass, and mycelium	9,38,40, 48–54
S. henetus	Pellets and fragmented mycelium to fragmented mycelium	9
S. jomonijinensis	Pellets and disperse mycelium to disperse mycelium	9
S. kanamyceticus	Pellets to disperse mycelium	9
S lavendulae	Pellets to mycelial aggregates and mycelium	9
S. lipmanni	Pellets and disperse mycelium	9
S. peuciticus	Pellets and mycelial aggregates, and pellets to mycelial fragments	9,55
S. platensis	Pellets to mycelial aggregates	9
S. rimosus	Pellets to fragmented mycelium	11
S. rimosus var. *paromycinus*	Pellets to disperse mycelium	9
S. rubrimeticuli	Pellets to pellets and mycelial aggregates	9
S. venezuelae	Pellets, mycelium	48,56,57
S. vinaceus	Pellets (nonproducer), and mycelium (antibiotic producer)	8
S. viridochromogenes	Pellets to disperse mycelium, and pellets	9 58

Table 2
Growth Forms in Fermenters and Other Stirred Vessels

Strain	Morphological form	Culture vessel	Reference
Actinomyces israelli	Pellets	Stirred anaerobic	59
Nocardia mediterranei	Mycelium	Fermenter	60
N. rhodocrous	Pseudomycelium to fragments; clumps at high, dissolved oxygen tension	Fermenter	61
Streptomyces achromogenes	Filamentous mycelium	Continuous culture	62
S. aureofaciens	Filamentous mycelium	Continuous culture	63,64
S. cattleya	Mycelium	Magnetically stirred flask	65
S. erythreus	Pellets	Fermenter	66,67
S. hygroscopicus	Small pellets	Continuous culture	68
S. lavendulae	Mycelial aggregates to mycelium	Continuous culture	63
	Mycelium	Range of fermenters	69
S. niveus	Mycelium	Continuous culture	62
	Mycelial aggregates	Tower fermenter	35
	Disperse mycelium	Fermenter	70
S. rectus var. *proteolyticus*	Mycelium	Fermenter	71
S. venezuelae	Mycelial aggregates or mycelial fragments	Fermenter	56
Thermomonospora sp.	Fragmented growth	Deep jet fermenter	72

found that cultures grown from low inocula tended to produce pellets rather than disperse mycelium. Biomass yield was proportional to inoculum size at spore concentrations between 3.7×10^4 and 8.7×10^5 spores mL^{-1}. However, actinorhodin was only produced at inoculum sizes greater than 1×10^5 spores mL^{-1}.

Vecht-Lifshitz et al. found that the average pellet size of *S. tendae* seemed to be inversely proportional to the order of magnitude of the inoculum size (*14*). There was, however, an inoculum size range where the average pellet size reached a plateau. With 10^7–10^{10} spores/m^3, the average pellet size remained approx 1 mm in diameter.

Influence of Media

Disperse growth is more likely in rich, complex media, and pellets occur in chemically defined media. However, effects of media can be extremely varied, and individual components may only change the morphological state of a few strains.

The nitrogen source influences the pellet form of *S. bambergiensis* (*12*). Casein hydrosylate led to the formation of small, firm, smooth pellets; whereas fodder feed yeast led to large, dishevelled pellets, and corn steep liquor led to soft pellets with different lengths of hyphae on the surface. The addition of butyrolactone derivatives to pellet-forming mutants of *S. griseus* led to the formation of long, filamentous hyphal aggregates characteristic of the parental strains (*50*).

In a few instances, a filamentous form is obtained in a complex medium and a more fragmented form in a defined medium. *Streptosporangiu viridogriseum* formed filamentous clumps in a complex medium containing starch but grew as fragmented mycelium in a defined sucrose asparagine mineral medium (*76*). *Streptomyces granticolor* was mycelial in media containing beef extract but fragmented when the nitrogen source was amino acids and nucleic acid bases or when glucose was omitted as a carbon source (*77,78*). In contrast, *S. venezuelae* formed hyphal microcolonies in glucose mineral salts and a glycerol serine lactate medium and fragmented in glucose yeast extract nutrient broth (*56*). Shomura et al. found that *S. halstedii* was affected by medium component concentration (*79*). It fragmented in concentrated media and retained a mycelial form in dilute media.

Influence of Culture Vessels on Growth Forms

The type of shaker may affect the growth form of a few actinomycetes. Cultures of *S. galileus* and *S. glomeratus* E65 and 65/22 grew as pellets on a reciprocal shaker and disperse mycelium on a rotary shaker (*45,47*). However, *S. glomeratus* C51e and C54 grew as pellets on both types of shaker.

In fermenters, pellets do not normally form because of the high aeration and vigorous agitation (*see* also Table 2): Filamentous mycelial growths are the predominant growth form (*56,60,62,71*). When *S. rectus* var *proteolyticus* was grown in batch culture, the mycelium was markedly shorter at 500–600 rpm than at 300–400 rpm. High agitation rates were thought to cause the shear of mycelium, the inhibition of elongation, or both (*71*). *Nocardia rhodocrous,* which normally grows as pseudomycelium or mycelial fragments in a fermenter, clumped at high, dissolved oxygen tensions (*61*). However, even at high stirring rates (3000 rpm), *S. hygroscopicus* still formed small mycelial pellets with 20–30 branching tips in continuous

culture with a glass blade stirrer, and *S. erythreus* grew as small pellets at 1000 rpm (*66–68*). *S. niveus* changed from a mycelial form in batch culture to a particulate aeratable form in continuous culture when growing in a tower fermenter (*35*).

Ways to Prevent Pelleting

Production of disperse mycelium is normally desirable in biochemical, physiological, or genetic studies because it tends to be more homogenous than mycelium tightly packed in a pellet. Dispersion is usually obtained by using modified shake flasks, maceration, adding fragmenting structures, altering the medium components or concentrations, using chemical additives, or combinations of these techniques.

Kominek used a Waring blender for 3 min to obtain disperse inocula of *S. griseus* and *S. niveus* (*80,81*). The blending led to decreases in incubation time to reach maximum growth, higher cell yields, and increased antibiotic titer. The same technique was used with *S. tenebrarius* in a study of nebramycin production (*82*). This technique is not easy to do aseptically and is very labour intensive on a multiple scale.

Baffled (cleated) flasks have been used to produce a more open mycelial morphology (*83,84*). However, these flasks cause splashing at high shaker speeds and tend to make filamentous fungi and actinomycetes stick to the walls of flasks above the level of the medium (*85*). Vecht-Lifshitz et al. have reported that pellet formation of *S. tendae* in baffled flasks was markedly decreased above inoculum sizes of 10^9 spores/m^3, and only a few small 0.5mm pellets were formed (*86*). In unbaffled flasks, the upper level for pellet formation was 10^{11} spores/m^3.

Toyama et al. used stainless steel coils in shaken culture to prevent the formation of pellets with 19 *Streptoverticillium* sp and seven *Streptomyces* sp (*87*). Similar techniques have been used with *S. coelicolor* and *S. peuciticus* (*55,88*).

Meshkov and Levitov used glass beads to produce diffuse mycelial growth of *S. fradiae* in a synthetic medium (*89*). The culture produced more neomycin (1800–2000 γ/cm^3) than the pelleting culture (340 γ/cm^3) growing without beads. Ochi prevented clumping of *S. coelicolor* by initially preparing a monospore suspension in a tube containing 3mm glass beads and blending on a vortex mixer for 2 min (*90*). Glass beads were used by Asai et al. to simulate the mechanical damage found in a 200 dm^3 fermenter during a fermentation of *S. hygroscopicus* for the production of maridomycin (*91*). Miyoshi et al. used glass beads and glass rods to exert mechanical forces on an improved strain of *S. sapporenensis* that produces bicyclomycin (*92*). However, these conditions resulted in a high proportion of low-producing isolates.

Anionic polymers, such as, carbopol (carboxypolymethylene) and Junlon PW 110 (a polyacrylic acid), have also been used to obtain disperse mycelium. These compounds are thought to affect the surface charges of spores and therefore reduce their agglutination potential. Hobbs et al. found that carbopol and Junlon PW 110 increased dispersion and cell yields of *S. lividans* and *S. coelicolor* (*16*). The presence of Junlon also stimulated the production of actinorhodin by *S. coelicolor*.

Submerged Sporulation

Most actinomycetes do not sporulate in submerged culture (*73,74*). Among the strains that do, it is important to distinguish between those that form spores similar to the ones produced on aerial mycelium and others that produce arthrospores intracellularly from mycelial cells. Although still not a common phenomenon, submerged sporulation has been observed in cultures of *Streptomyces acrimycini, S. albus, S. coelicolor, S. griseus, S. lavendulae, S. roseosporus, S. venezuelae, S. viridochromogenes,* and *Actinomyces* sp. (*10,38,75–82*).

Many strains of *S. griseus* and other species will form spores in 1–4 d depending on the strain and the medium, but the quantity of spores produced may vary. This type of spore formation is most often associated with a nutritional downshift that results from depletion of an essential nutrient, often nitrogen or phosphate (*75,77,79*). An excess of the nitrogen source inhibits spore formation of cultures of *S. venezuelae* (*82*). *S. griseus* NRRL B2682 was not affected by variations in medium concentration (*80*). However, if the initial spore concentration in the medium was increased to 1×10^8 spores/cm^3, no spores were produced within 7 d. A stable mutant (bald 10) would form spores if A-Factor was present in the medium. The formation of submerged spores of *S. roseosporus* did not occur in either a primary or a secondary medium but only in a tertiary fermentation medium that contained methyl oleate as a carbon source and was not due to nutrient depletion.

Tresner et al. found that a number of *S. aureofaciens* strains produced arthrospore-like cells after growth in AC Difco broth for 3 d (*7*). *Streptomyces virginae* formed arthrospores after 2 d when glucose had been exhausted in the growth medium.

Influence of Morphology on the Ability of Actinomycetes to Produce Antibiotics and Other Products in Submerged Culture

A number of workers have attempted to identify morphological forms that are associated with production of antibiotics or other metabolites. A disperse mycelial form that is not starved of oxygen or nutrients is nor-

mally considered ideal for industrial fermentations, but very little specific published information is available (*103,104*).

A mycelial form of *S. griseus* is required for streptomycin production, but pelleted and fragmented forms are undesirable (*51,53,96*). Other strains that fragment quickly without clumping were also nonproducing (*95*). The filamentous state is also essential for production of antibiotic SF-1993 by *S. halstedii* and turimycin by *S. hygroscopicus* (*68,79*). In both cases, no antibiotic was produced by the fragmented form. Pelleted growth of *Nocardia lactamdurans* in a seed fermenter led to depressed production of cephamycin in a subsequent production stage (*96*).

Lower streptomycin production was also associated with increased submerged spore production by *S. griseus* (*95,105*). Arthrospore production by *S. virginiae* inhibited the production of virginiamycin (*107*). However, in an *Actinomyces* sp. culture, the release of spore-like bodies was associated with the production of an insecticidal antibiotic. The absence of spores was associated with initial and late production of an antibiotic active against gram-positive bacteria (*10*).

Pellets of *S. nigrificans* produced more glucose isomerase than the mycelial form (*108*). The same enzyme has also been isolated from the pelleted form of *S. bambergiensis* (*12*).

Braun and Vecht-Lifshitz have concluded that the effects of pellet morphology on productivity and composition of secondary metabolites remain unexplained (*104*). It may therefore be possible to obtain specific metabolites not synthesized by disperse mycelial growth.

REFERENCES

1. Whitaker, A. and Long, P. A. (1973), *Proc. Biochem.* **8(11),** 27.
2. Metz, B. and Kossen, N. W. F. (1977), *Biotech. Bioeng.* **19,** 781.
3. Adams, H. L. and Thomas, C. R. (1988), *Biotech. Bioeng.* **32,** 707.
4. Erikson, D. and Porteous, J. W. (1955), *J. Gen. Microbiol.* **13,** 464.
5. Waksman, S. A. (1950), *The Actinomycetes: Their Nature, Occurrence, Activities and Importance,* Chronica Botanica Co., Waltham, MA, pp. 46.
6. Waksman, S. A. (1959), *The Actinomycetes: Nature, Occurrence and Activities,* vol. *1,* Balliere, Tindall and Cox, London, pp. 113.
7. Tresner, H. D., Hayes, J. A., and Backus, E. J. (1967), *Appl. Microbiol.* **15,** 1185.
8. Williams, S. T., Entwistle, S., and Kurylowicz, W. (1974), *Microbios* **11A,** 47.
9. Lawton, P., Whitaker, A., Odell, D., and Stowell, J. D. (1989), *Can. J. Microbiol.* **35,** 881.
10. Blumauerova, M., Wolf, A., Jizba, J., Vankova, J., Stastna, J., and Vanek, Z. (1985), *6th International Symposium on Actinomycete Biology.* Szabo, G., Biro, S., and Goodfellow, M., eds., pp. 855.
11. Al-Jawadi, M. and Calam, C. T. (1987), *Folia Microbiol.* **32,** 388.

12. Cizmek, S. and Drazic, M. (1987), *Proceedings of the 4th European Congress on Biotechnology,* Neijssel, O. M., van der Meer, R. R., and Luyben, K. C. A. M., eds., vol. 2, Elsevier, Amsterdam, pp. 233.
13. Hobbs, G., Frazer, C. M., Gardner, D. C. J., Flett, F., and Oliver, S. G. (1990), *J. Gen. Microbiol.* **136,** 2291.
14. Vecht-Lifshitz, S. E., Magdassi, S., and Braun, S. (1990), *Biotech. Bioeng.* **35,** 890.
15. Kristufek, V. and Blumauerova, M. (1983), *Folia Microbiol.* **28,** 179.
16. Hobbs, G., Frazer, C. M., Gardner, D. C. J., Cullum, J. A., and Oliver, S. G. (1989), *Appl. Microbiol. Biotech.* **31,** 272.
17. Douglas, R. J. and San Clemente, C. L. (1956), *Can. J. Microbiol.* **2,** 407.
18. Bradley, S. G. (1962), *Dev. Ind. Microbiol.* **3,** 362.
19. Tagawa, K. and Okazaki, K. (1991), *J. Ferm. Bioeng.* **71,** 230.
20. Gusek, T. W., Johnson, R. D., Tyn, M. T., and Kinsella, C. (1991), *Biotech. Bioeng.* **37,** 371.
21. Ishiguro, E. E. and Wolfe, R. S. (1970), *J. Bact.* **104,** 566.
22. Woycha, M. G. and Biggs, C. B. (1982), *US Patent 4,345,030.*
23. Watanabe, S., Kumagaya, A., and Murooka, H. (1963), *J. Gen. Appl. Microbiol.* **9,** 363.
24. Beaman, B. L. (1975), *J. Bact.* **123,** 1235.
25. Adams, J. A. (1966), *Can. J. Microbiol.* **12,** 433.
26. Lefebvre, G., Martin, N., Kilbertus, G., and Gay, R. (1978), *J. Gen. Microbiol.* **108,** 125.
27. Michaels, G. E., Eagon, R. G., and McClung, N. M. (1960), *Bact. Proc.* 85.
28. Hopwood, D. A., Wright, H. M., Bibb, M. J., and Cohen, S. N. (1977), *Nature* **268,** 171.
29. Manis, J. J. and Highlander, S. K. (1982), *US Patent 4,332,900.*
30. Thirumalachar, M. J., Rahalkar, P. W., and Deshmukh, P. V. (1971), *Hindustan Antibiotics Bull.* **13,** 67.
31. Stastna, J. and Caslavska, J. (1976), *Folia Microbiol.* **21,** 203.
32. Baltz, R. H. (1978), *J. Gen. Microbiol.* **107,** 93.
33. Grafe, U. Reinhardt, G., Krebs, D., Roth, M., and Noack, D. (1982), *J. Gen. Microbiol.* **128,** 2693.
34. Hall, M. J. and Hassall, C. H. (1970), *Appl. Microbiol.* **19,** 109.
35. Ross, N. G. and Wilkin, G. D. (1968), *British Patent 1,133,875.*
36. Stroman, D. W. and Demetrios, A. (1982), *British Patent Application GB 2,097,002 A.*
37. Dworschack, R. G., Chen, J. G., Laman, W. R., and Davis, L. G. (1973), *U.S. Patent 3,736,232.*
38. Gottlieb, D. and Anderson, H. W. (1947), *Bull. Torrey Bot. Club* **74,** 293.
39. Mahmoud, W. and Rehm, H. J. (1986), *Appl. Microbiol. Biotech.* **23,** 305.
40. Dickenson, P. B. and Macdonald, K. D. (1955), *J. Gen. Microbiol.* **13,** 84.
41. Scotti, T. and Zocchi, P. (1955), *Giornale di Microbiologia* **1,** 35.
42. Cochrane, V. W. and Peck, H. D. (1953), *J. Bact.* **65,** 37.
43. Hodgson, D. A. (1982), *J. Gen. Microbiol.* **128,** 2417.
44. Corum, C. J., Stark, W. M., Wild, G. M., and Bird, H. L. (1954), *Appl. Microbiol.* **2,** 326.
45. Kralovcova, E. and Vanek, Z. (1974), *Folia Microbiol.* **24,** 301.

46. Blumauerova, M., Podojil, M., Gauze, G. F., Maksimova, T. S., Panos, J., and Vanek, Z. (1980), *Folia Microbiol.* **25,** 207.
47. Blumauerova, M., Podojil, M., Gauze, G. F., Maksimova, T. S., Panos, J., and Vanek, Z. (1980), *Folia Microbiol.* **25,** 213.
48. Gottlieb, D. and Legator, M. (1953), *Mycologia* **45,** 507.
49. Vitalis, G. S., Szabo, G., and Valyi-Nagy, T. (1963), *Acta Biol. Acad. Sci. Hung.* **14,** 1.
50. Grafe, U. (1984), *J. Gen. Microbiol.* **130,** 1237.
51. Schatz, A. and Waksman, S. A. (1945), *Proc. Nat. Acad. Sci.* **31,** 129.
52. Wilkin, G. D. and Rhodes, A. (1955), *J. Gen. Microbiol.* **12,** 259.
53. Szabo, G., Barabas, G., and Valyi-Nagy, T. (1961), *Arch. Mikrobiol.* **40,** 261.
54. Fortney, K. F. and Thoma, R. W. (1977), *Dev. Ind. Microbiol.* **18,** 319.
55. Dekleva, M. L., Titus, J. A., and Strohl, W. R. (1985), *Can. J. Microbiol.* **31,** 287.
56. Berwick, M. W. M., Williams, S. T., and Veltkamp, C. (1976), *Microbios* **16,** 191.
57. Jones, L. A. and Bradley, S. G. (1965), *J. Gen. Microbiol.* **40,** 191.
58. Lindenfelser, L. A. and Pridham, T. G. (1962), *Dev. Ind. Microbiol.* **3,** 245.
59. Christie, A. O. and Porteous, J. W. (1960), *J. Gen. Microbiol.* **23,** 261.
60. Lee, J. G., Choi, C. Y., Seong, B. L., and Han, M. H. (1983), *J. Ferm. Tech.* **61,** 49.
61. Buckland, B. C., Lilly, M. D., and Dunnill, P. (1976), *Biotech. Bioeng.* **18,** 601.
62. Means, C. W., Savage, G. M., Reusser, F., and Koepsell, H. J. (1962), *Biotech. Bioeng.* **4,** 5.
63. Bekhtereva, M. N. and Kolesnikova, I. G. (1961), *Mikrobiologiya* **30,** 351.
64. Sikyta, B., Slezak, J., and Herold, M. (1961), *Appl. Microbiol.* **9,** 233.
65. Bushell, M. E. and Fryday, A. (1983), *J. Gen. Microbiol.* **129,** 1733.
66. Bosnjak, M., Vrana, M., and Topolovec, V. (1976), *Abstracts of Papers. Fifth International Fermentation Symposium,* Dallweg, H., ed., West Kreuz-Druckerei und Verlag, Berlin and Bonn, p. 219.
67. Bosnjak, M., Topolovec, V., and Vrana, M. (1978), *J. Appl. Chem. Biotechnol.* **28,** 791.
68. Roth, M. and Noack, D. (1982), *J. Gen. Microbiol.* **128,** 107.
69. Ziegler, H., Dunn, I. J., and Bourne, J. R. (1980), *Biotech. Bioeng.* **22,** 1613.
70. Wang, D. I. C. and Fewkes, R. C. J. (1977), *Dev. Ind. Microbiol.* **18,** 39.
71. Mizusawa, K., Ichishima, E., and Yoshida, F. (1969), *Appl. Microbiol.* **17,** 366.
72. Meyer, H. P. and Charles, M. (1982), *Biotech. Bioeng.* **24,** 1905.
73. Kolstad, R. A. and Bradley, S. G. (1967), *Dev. Ind. Microbiol.* **8,** 198.
74. Erikson, D. (1955), *J. Gen. Microbiol.* **13,** 119.
75. Hirsch, C. F. and Ensign, J. C. (1976), *J. Bact.* **126,** 13.
76. Oh, Y. K. and Nash, C. H. (1981), *Folia Microbiol.* **26,** 164.
77. Stastna, J., Caslavska, J., Wolf, A., Vinter, V., and Mikulik, K. (1977), *Folia Microbiol.* **22,** 339.
78. Caslavska, J., Stastna, J., Ludvik, J., and Blumauerova, M. (1978), *Proceedings of the International Symposium on Nocardia and Streptomyces,* Mordarski, M., Kurylowicz, W., and Jeljaszewica, J., eds., Gustav Fischer Verlag, Stuttgart, p. 181.

79. Shomura, T., Yoshida, J., Amano, S., Kojima, M., Inouye, S., and Nida, T. (1979), *J. Antibiotics* **32,** 427.
80. Kominek, L. A. (1972), *Antimicrob. Agents Chemother.* **1,** 123.
81. Kominek, L. A. (1975), *Antimicrob. Agents Chemother.* **7,** 856.
82. Higgens, C. E. and Kastner, R. E. (1968), *Antimicrob. Agents Chemother.* **1,** 324.
83. Sebek, O. K. (1955), *Arch. Biochem.* **57,** 71.
84. Robertson, J. G. and Batt, R. D. (1973), *J. Gen. Microbiol.* **78,** 109.
85. Pirt, S. J. (1975), *Principles of Microbe and Cell Cultivation,* Blackwell, Oxford, p. 103.
86. Vecht-Lifshitz, S. E., Magdassi, S., and Braun, S. (1989), *J. Dispersion Sci. Tech.* **10,** 265.
87. Toyama, H., Okanishi, M., and Umezawa, H. (1974), *J. Gen. Microbiol.* **80,** 507.
88. Wheelis, W. H. and Wells, B. (1974), *John Innes Ann. Rep.* 77.
89. Meshkov, A. N. and Levitov, M. M. (1965), *Antibiotiki* **10,** 685.
90. Ochi, K. (1986), *J. Gen. Microbiol.* **132,** 2621.
91. Asai, T., Sawada, H., Yamaguchi, T., Suzuki, M., Higashide, E., and Uchida, M. (1978), *J. Ferm. Tech.* **56,** 374.
92. Miyoshi, T., Iseki, M., Konomi, T., and Imanaka, H. (1980), *J. Antibiotics* **33,** 480.
93. Kalakoutskii, L. V. and Agre, N. S. (1976), *Bact. Rev.* **40,** 469.
94. Ensign, J. C. (1978), *Ann. Rev. Microbiol.* **32,** 185.
95. Daza, A., Martin, J. F., Dominguez, A., and Gil, J. A. (1989), *J. Gen. Microbiol.* **135,** 2483.
96. Carvajal, F. (1947), *Mycologia* **39,** 426.
97. Koepsel, R. and Ensign, J. C. (1984), *Arch. Microbiol.* **140,** 9.
98. Vitalis, G. S., Biro, S., Vargha, G., Bekesi, I., and Szabo, G. (1981), *Actinomycetes: Zbl. Bakt. Suppl. 11,* Schaal, K. G. and Pulverer, G., eds., Gustav Fischer Verlag, Stuttgart.
99. Kendrick, K. E. and Ensign, J. C. (1983), *J. Bact.* **155,** 357.
100. Ensign, J. C., McBride, M. J., Stoxen, L. J., Bertinuson, A., Pomplum, M., and Ho, A. (1985), *6th International Symposium on Actinomycete Biology,* Szabo, G., Biro, M., and Goodfellow, M., eds., p. 777.
101. Huber, F. M., Piper, R. L., and Mertz, F. P. (1987), *J. Indust. Microbiol.* **2,** 235.
102. Glazebrook, M. A., Doull, J. L., Stuttard, C., and Vining, L. C. (1990), *J. Gen. Microbiol.* **136,** 581.
103. Vandamme, E. J. (1984), *Biotechnology of Industrial Antibiotics,* Marcel Dekker, Basel.
104. Braun, S. and Vecht-Lifshitz, S. E. (1991), *Trends in Biotechnol.* **9,** 63.
105. Nomi, R. (1963), *Appl. Microbiol.* **11,** 84.
106. Ohmstead, D. R., Hunt, G. R., and Buckland, B. C. (1985), *Comprehensive Biotechnology,* vol 3, Blanch, H. W., Drews, S., and Wang, D. I. C., eds., Pergamon, Oxford, 187.
107. Biot, A. M. (1984), *Biotechnology of Industrial Antibiotics,* Vandamme, E. J., ed., Marcel Dekker, Basel, p. 695.
108. Demnerova, K., Marek, M., and Kralova, B. (1984), *Folia Microbiol.* **29,** 423.